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TIDAL RHYTHMS OF ACTIVITY AND PHOTOTAXIS OF AN ESTUARINE CRAB LARVA

RICHARD B. FORWARD, JR., AND THOMAS W. CRONIN[1]

Duke University Marine Laboratory, Beaufort, N.C. 28516 and Zoology Department, Duke University, Durham, N.C. 27706

In estuaries, the net flow of water is seaward. As a consequence, estuarine plankton continually risk export into adjacent coastal waters. This risk is especially severe among many of the relatively short lived planktonic larvae of benthic invertebrates, whose survival depends upon being in the estuary near a suitable adult habitat at metamorphosis.

Larvae of the estuarine crab *Rhithropanopeus harrisii* are retained in the estuary near the adult population throughout development (Cronin, 1979; Sandifer, 1973, 1975). Cronin (1979) studied the vertical distribution of *R. harrisii* larvae in an area having pronounced semi-diurnal tides and found that all larval stages usually occur near the depth of no net motion. Although this generally accounts for their retention near the parent population, larvae do not continually stay at the depth of no net motion. Rather, they migrate vertically around this depth. These movements are related to environmental cycles in light and dark, salinity and current speed (Cronin, 1979). Further laboratory studies indicate that the larvae have a pronounced endogenous tidal vertical migration pattern in which the maximum depth is reached around the time of low tide and minimum at high tide (Cronin and Forward, 1979).

The present study was initiated to investigate the behavioral basis of these vertical migration rhythms in *R. harrisii* larvae. Swimming speed and light oriented movement (phototaxis) were monitored in freshly caught larvae. These types of behavior were chosen because vertical movements may result from changes in activity, and phototaxis may contribute to daily migrations relative to the natural light : dark cycle. We found endogenous tidal rhythms in both swimming speed and phototaxis.

Materials and Methods

Larvae from the crab *Rhithropanopeus harrisii* (Gould) were collected with a No. 2 plankton net in the Newport River estuary, North Carolina. All collections were during daytime high tides in water of about 16‰ salinity at depths of about 0.5 to 1 m. Larvae were taken rapidly to the laboratory, where stage III zoeae were sorted out for experimentation. This stage was used because it has a pronounced endogenous rhythm in tidal vertical migration (Cronin and Forward, 1979). Groups of larvae were placed in 11.4-cm-diameter finger bowls in water from the collection site, which was filtered to remove particles larger than 5 μ. Thereafter, the larvae were maintained under constant conditions of light (cool white fluorescent lamp; intensity of 19 W m^{-2}), salinity, and temperature (30° C $\pm$ 0.5°) in a Sherer controlled environmental chamber (model CEL 4–4). This temperature

[1] Present address: Biology Department, 260 Whitney Ave., Yale University, New Haven, CT 06520.

was used because water temperatures at the collection site ranged around 30° C.

The rhythm in speed of swimming was measured on two occasions (beginning dates July 24 and 30, 1979). The time of tides had advanced about 4 hr from the first date to the second. In both cases, three groups of 50 larvae each were placed in the controlled environmental chamber. At 2-hr intervals, all of the larvae from each bowl were placed in a lucite cuvette on the stage of a dissecting microscope and observed by means of a closed-circuit television system (Forward, 1974a; Forward and Costlow, 1974). The microscope illumination was filtered to the infrared (IR) region (American Optical Co. Filter No. 775–752). The larvae are insensitive to wavelengths in this region (Forward and Costlow, 1974).

The exact procedure was to place the 50 larvae on the microscope stage for 1 min in darkness, and then record swimming on video tape for 30 to 45 sec. Larvae were returned to the controlled environmental chamber, placed in new water and given a small amount of *Artemia salina* nauplii. In this way the larvae were placed in new water and given new food every 2 hr. Newly hatched *A. salina* nauplii were obtained each morning and used throughout the day. This procedure maintained the larvae in a viable condition, as indicated by the fact that an average of 89% (SE = 1%) of the larvae were alive at the end of each experiment. Swimming speeds of 15 larvae chosen arbitrarily from each group were analyzed using methods previously described (Forward and Costlow, 1974).

Phototaxis was measured by light stimulation in the horizontal plane in order to avoid complications due to directional responses to gravity (geotaxis). The stimulus source was a slide projector having a 300 W incandescent bulb. The light was filtered by two hot mirrors (Baird Atomic) and a Corning No. 1–75 IR absorbing filter in order to remove heat and was then filtered to 500 nm with an interference filter (Optics Technology; half band pass 14 nm). The larvae are maximally sensitive to wavelengths in this region (Forward and Costlow, 1974). The light intensity was regulated by neutral density filters and was measured with a radiometer (EG and G, model 550).

The test chamber consisted of a rectangular lucite cuvette (15 × 3 × 3 cm), which was divided into five equal sections along the longitudinal axis. The sections were separated by thin partitions constructed so that all could be moved vertically in unison. For determining phototactic responsiveness, a group of animals was placed in the center section of the cuvette. The animals were left 1 min in darkness, the partitions separating the sections were raised, the animals were stimulated for 30 sec, and then the partitions were returned. The distribution of animals among the five sections was then recorded. Control responses were determined by following the same procedure, except that animals remained in darkness for the entire trial. For establishing phototactic response levels, animals located in the two sections nearest the light source were considered to display positive phototaxis, while those in the furthest two sections were considered negative.

There were two series of phototactic experiments. In the first, responsiveness to a range of stimulus intensities was measured shortly before high and low tides. Larvae were collected at high tide in the morning on two consecutive days, taken rapidly to the laboratory, sorted into seven groups of 10 larvae each, and maintained in a light-adapted state in water from the collection site, under the conditions described above in the constant environmental chamber. Beginning an average of 45 min before the subsequent low tide, phototactic responsiveness to a range of

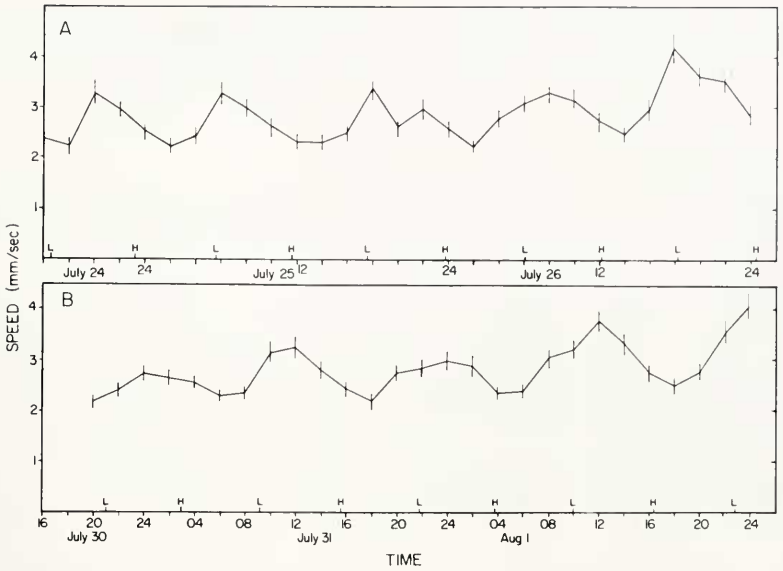

FIGURE 1. Rhythm in swimming speeds (ordinate) over time (abscissa) for larvae collected on July 24 (A) and July 30, 1979 (B). Mean speeds and standard error of 45 determinations are shown. L is time of low tide, and H shows high tide.

intensities was measured. Each group of larvae was tested at two different intensities. Larvae were then maintained in the environmental chamber until about 45 min before the next high tide, when photoresponsiveness was again tested. Tidal times were established by adjusting the NOAA tide table times for Pivers Island, North Carolina, to a predicted time at the collecting site in the Newport River estuary (+ 1.5 hr).

The second experimental series involved determining the rhythm in phototaxis. Larvae were collected on two occasions (August 6 and 15, 1979). Tidal times advanced about 8 hr between the two runs. Larvae were sorted into three groups of about 30 individuals each and placed under constant conditions. At 2-hr intervals, phototaxis by larvae in each bowl was tested using the described procedure. After phototactic responsiveness was tested, the larvae were placed in new water from the collection site and fed *A. salina* nauplii.

RESULTS

Rhythm in swimming speeds

The larvae have a clear endogenous tidal rhythm in swimming speeds (Fig. 1). In both trials (Fig. 1A, B) using the Fisher periodogram test (Fuller, 1976) the frequency nearest the tidal cycle is significant ($P < 0.05$). The speeds increase to a maximum within 3 hr after low tide, and then decrease to a minimum about 2 hr after high tide. This pattern is seen on both test dates and is related to tidal times. This is indicated by the fact that both the time of tides and of rhythmic swimming advance by 4 hr from trial 1 (Fig. 1A) to trial 2 (Fig. 1B).

Speeds vary between similar maximum and minimum values over about the first 36 hr. The final maximum values are much higher. This reflects the molt

of most larvae to stage IV zoeae which swim faster than stage III (Forward and Costlow, 1974). Even with this molt, the rhythm continues under constant conditions for at least 54 hr. There is no indication of a diel component of the rhythm.

Phototaxis

Generally the larvae respond positively to high light intensities and negatively to low (Fig. 2), which is the typical pattern (Forward and Costlow, 1974). The curves of responsiveness to different light intensities suggest that phototaxis varies between high and low tide (Figs. 2A, B). Mean positive response is always lower at high tide than low tide (Fig. 1A), but the curves at each phase of the tide are not significantly different (two way ANOVA). The opposite pattern occurs for negative phototaxis (Fig. 2B) in which at high tide responsiveness is greater at intensities of about 10^{-4} to 10^{-3} W m^{-2}. The curves at each tide are not significantly different but at 8.5×10^{-3} W m^{-2} the overall response levels are different ($P < 0.02$; Z statistic for comparing proportions).

These results suggest that phototaxis varies with phase of the tide. The magni-

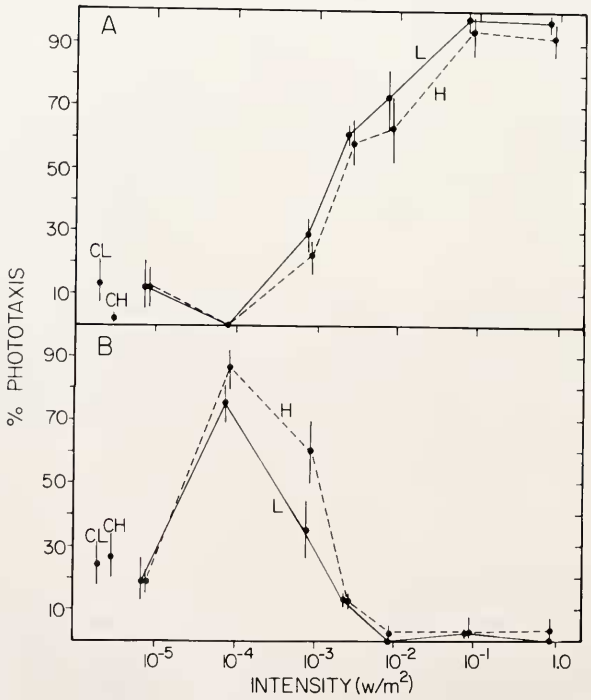

FIGURE 2. Per cent positive (A) and negative (B) phototaxis (ordinate) upon stimulation with a range of light intensities (abscissa). Mean percentages plus standard error are shown. Four trials were conducted with about 30 individuals in each trial. The dashed lines are for high tide (H) and the solid line for low tide (L). Stimulus intensities were the same at both tides, but for clarity, points are slightly offset in plotting. C indicates the control levels at the different tides.

tude of the variation is probably greater than shown because high and low tides were arbitrarily chosen as the time for measurements. Since rhythms in phototaxis are most easily demonstrated by measuring response variation over time upon stimulation with a single intensity, Figures 2A and 2B are useful for indicating the proper intensity range. The values chosen for stimulation are between 6.6×10^{-3} and 1.4×10^{-2} W m^{-2} because these intensities are non-saturating and fall in the area where the sign of phototaxis is changing.

Although Figure 3 shows a rhythmic change in phototaxis, the Fisher periodogram test does not indicate a significant frequency. This is probably due to the rhythm being measured over a relatively short time, showing consistent periodicity early in the records and being less regular in later measurements.

The general pattern is one in which negative phototaxis rises to its greatest level around the middle of the rising tide and declines to a minimum during falling tides. Positive phototaxis has an opposite pattern of greatest responsiveness during falling tides and lowest on rising tides. The rhythm is more pronounced in the second trial (Fig. 3B), probably because a lower stimulus light intensity was used. Clearly, the rhythm is related to natural tidal times, since the timing of the rhythm advances by about the same time (8 hr) as tidal times change from the first to second test dates. Furthermore, the rhythm is endogenous, since it persists under constant conditions for at least 40 hr. The pattern is not as pronounced after this time.

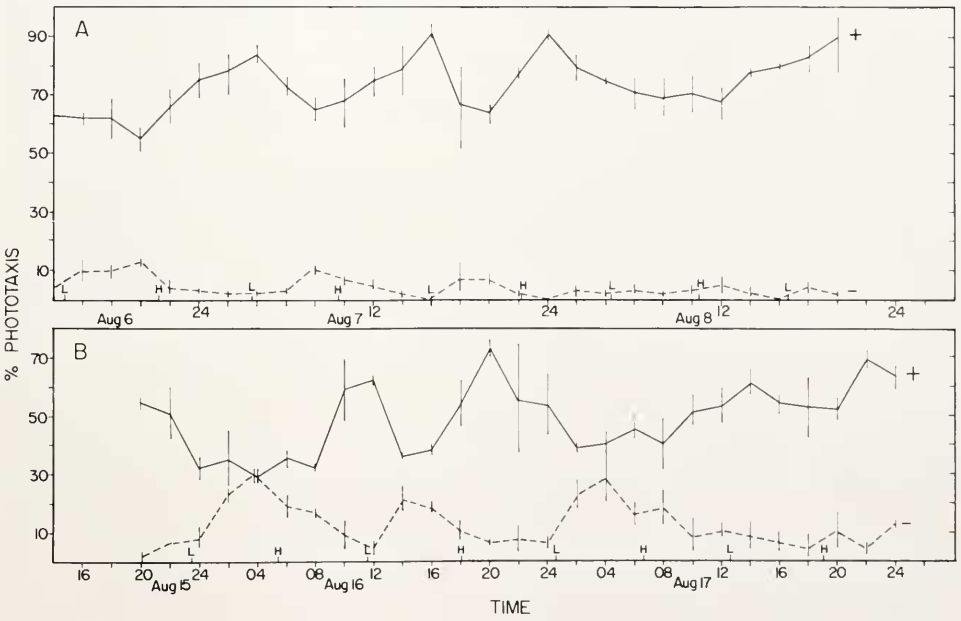

Figure 3. Rhythm in positive (solid line) and negative (dashed line) phototaxis (ordinate) over time (abscissa) for larvae collected on August 6, 1979 (A) and August 15, 1979 (B). Mean and standard error are shown at each time. Three trials were conducted with about 30 individuals in each trial. L is time of low tide, and H is time of high tide. The stimulus intensity in A was 6.6×10^{-3} W m^{-2} until 0200 on August 7 when it increased to 1.4×10^{-2} W m^{-2} as the lamp was replaced. In B the stimulus intensity was 7.4×10^{-3} W m^{-2}.

Discussion

Many intertidal organisms have tidal rhythms in behavior, physiology, and reproduction (reviewed by Palmer, 1973). Until the recent demonstration of an endogenous tidal vertical migration of *R. harrisii* larvae (Cronin and Forward, 1979), tidal rhythms in plankton were unreported. The present study presents further evidence of endogenous tidal rhythmicity in larvae of this species. The presence of tidal rhythms in an estuarine plankter is perhaps not surprising, since estuaries frequently have pronounced tidal currents, and the varying behavior may allow the animals to exploit different phases of the tide. Thus it may be constructive to consider the possible functions of the rhythms in swimming speed and phototaxis of *R. harrisii* larvae.

In studies of tidal rhythms, the activity patterns of organisms have perhaps been most often measured (Palmer, 1973). The rhythmic changes in swimming speed (Fig. 1) displayed by *R. harrisii* larvae represent an activity rhythm. The pattern shows highest activity during rising tides, with a maximum usually several hours after low tide, and lowest activity during falling tides, with the minimum occurring several hours after high tide. This pattern could be the basis of the tidal rhythm in vertical migrations observed in the laboratory (Cronin and Forward, 1979).

In the laboratory-observed migrations, the larvae descend to a maximum depth at about the time of low tide and rise to a minimum depth at approximately high tide. The larvae are actually migrating over a short vertical distance of 1 to 1.5 m. The minimum time for the ascent and descent over this distance is 3 hr (Cronin and Forward, 1979). Since the minimum swimming speed observed in the rhythm study (Fig. 1) is about 2.25 mm/sec or 8.1 m/hr, it is obvious that larvae are not swimming directly up or down. It is also doubtful that the larvae are only sinking during the descent, since stage IV zoeae passively sink at a rate of 33 m/hr (15‰ salinity, 23–25° C; Latz and Forward, 1977), which far exceeds the rate of descent.

Alternatively, their depth could change with alterations in activity. If we assume that larvae migrate about some constant depth and that swimming speeds contribute to the direction of migration, then it is reasonable to postulate that at the average swimming speed, the larvae are just maintaining a constant depth in the water. Thus, when the swimming speeds are above the average, the larvae would rise, and, conversely, when swimming speeds are below the average, descent would occur. It is noteworthy that these average swimming speeds occur at times very near the tidal extremes (Fig. 1), and this corresponds closely to the times at which the migrations in constant conditions in the laboratory are at their limits (Cronin and Forward, 1979). Following low tide, the speeds are above average, which leads to a rise. The ascent decreases to zero near the time of high tide, when the average speed is again reached. After this, speeds are below average and the descent to the low tide depth would again occur. Thus the changes in swimming speed can lead to the laboratory-observed tidal vertical migration pattern.

The tidal vertical migration pattern observed in the field shows variable timing (Cronin, 1979). During spring tides when the water column is well mixed, larvae reach a minimum depth about 3 hr before the salinity maximum. On neap tides when more stratified conditions exist, larvae reach minimum depth 4.5 hr after the salinity maximum. Even though the activity rhythm (Fig. 1) was measured at the time of both neap and spring tides, it is very similar on both occasions. Considering the variable timing of the field tidal rhythms (Cronin,

1979) any relation between the activity rhythm in the laboratory and field observations is perhaps fortuitous. Nevertheless, if the activity rhythm is the basis for tidal migration in the field, then a pattern similar to that at spring tide would be predicted.

The activity pattern and associated tidal vertical migration would be adaptive for retention in estuaries. Stratified partially mixed estuaries have a layer of high salinity water with a net landward flow which underlies an upper layer of seaward-flowing lower-salinity water (Pritchard, 1952). If an animal partitions its time between these layers, it is possible to reduce displacement from the parent population. The observed tidal migration would serve this purpose. On rising tides activity would increase, and larvae would move up in the water column and be transported up the estuary. As the tide falls, activity is reduced and larvae descend. Seaward transport would be reduced because the slowest seaward flowing currents occur at greater depths.

Tidal rhythms in phototaxis among animals are not widely studied, and the only reported case is for a sand beach amphipod (Forward, 1980). In *R. harrisii* the rhythm consists of an increase in negative phototaxis to its greatest level around the middle of the rising tide and a decrease to a minimum during falling tide. The opposite pattern is seen for positive phototaxis.

Since phototaxis involves both sensory perception and directional swimming, it is possible that the phototactic rhythm simply results from the activity rhythm. This seems unlikely because the phototactic rhythm consistently continues for only 40 hr while the activity rhythm is still present after 54 hr. Furthermore, if the changes in activity cause alteration in phototaxis, then the overall level of both positive and negative phototaxis should cycle together as responsiveness increases (activity increase) and decreases. This does not occur (Fig. 3).

A more plausible interpretation is that the phototactic pattern is actually changing rhythmically. This is suggested by the data shown in Figure 2. The field captured larvae are light adapted and show the typical phototactic pattern (Forward and Costlow, 1974) of laboratory reared larvae. This consists of a positive response to high light intensities which becomes negative at low intensities. Positive phototaxis is consistently but not significantly lower and negative phototaxis significantly greater at the end of the rising tide (high tide) than at the end of the falling tide (low tide). The difference in phototaxis would be greater if measurement were made at times of greatest negative and positive responsiveness. In the rhythm experiments the stimulus intensity range chosen (10^{-3}–10^{-2} W m^{-2}) evokes both positive and negative phototaxis.

The sign of phototaxis varies rhythmically in that on rising tides negative phototaxis increases and the positive response decreases, with the opposite occurring on falling tides. Thus a discussion of functional significance must consider the change in the phototactic pattern over the tidal cycle.

The phototactic response pattern by *R. harrisii* larvae varies with light and dark adaptation and the light stimulus intensity. Upon light adaptation, the larvae are positive to moderate and high light intensities but become negative to lower intensities. The pattern reverses upon dark adaptation. Forward (1974b) originally hypothesized that the changes in phototaxis upon light and dark adaptation could lead to a diurnal vertical migration pattern involving an ascent at sunrise and descent at sunset. After considering larval photophysiology and rates of light intensity change underwater, Forward and Cronin (1978) concluded that it is very unlikely that the changing phototaxis patterns could

result in diurnal vertical migration. Furthermore, Cronin's (1979) field study demonstrated that larvae are generally nearer the surface at night than during the day, which is the reverse of the predicted diurnal vertical migration pattern.

Alternatively, phototaxis may participate in the tidal vertical migration. A generalized pattern based on the activity rhythm (Fig. 1), endogenous vertical movement (Cronin and Forward, 1979) and the field study of migration (Cronin, 1979) indicates that the larvae ascend on rising tides and descend on falling tides. It is questionable that phototaxis contributes to this migration because during the ascent there is an increase in negative and a decrease in positive phototaxis, while the opposite pattern occurs during the descent. Since positive response would lead to an ascent and negative to a descent, this rhythm in phototaxis would actually move the larvae in the opposite directions from those shown during tidal vertical migration. Thus there is no compelling evidence that phototaxis contributes to vertical migration by *R. harrisii* larvae.

Although speculation about the functional significance of diel vertical migration is extensive, perhaps the most common suggestion is predator avoidance. Zooplankton showing nocturnal and twilight migration patterns ascend into suface areas at times when illumination is low (night) and descend from these areas during the day, thereby avoiding visually oriented predators. In field studies, *R. harrisii* larvae primarily show a tidal migration pattern which has a diel component of shallower distributions at night than during the day (Cronin, 1979). This diel migration may also reflect predator avoidance. Nevertheless, because larvae migrate according to tides, they must move into shallow depths at some time during the day. In this area they would encounter visually oriented predators as well as those which swim slowly near the surface, such as ctenophores (See Forward, 1976, for a detailed discussion).

The most unusual aspect of larval phototaxis is the negative response to low light levels when light adapted. A series of studies (Forward, 1974b, 1976) supported the hypothesis that this negative phototaxis is part of a shadow response used for avoidance of predators which do not actively pursue their prey and occur close to the surface, such as ctenophores and coelenterate medusae.

The tidal rhythm in phototaxis shows an increase in negative responsiveness on rising tides. This is also the time the larvae ascend in the water column into better-illuminated areas. Thus, it is plausible to hypothesize that the functional significance of the phototactic rhythm is predator avoidance, in which the negative phototactic part of the shadow response is increased at times when the larvae are higher in the water column and thereby more likely to encounter ctenophores and coelenterate predators.

The present study presents further evidence of tidal rhythms in estuarine plankton. The occurrence of similar rhythms in other estuarine plankton and the aspects of the estuarine tidal cycle that actually entrain the rhythms remain to be determined.

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Summary

1. Tidal rhythms in swimming speed and phototaxis were measured in stage III zoeae of the crab *Rhithropanopeus harrisii*. Larvae were captured in the Newport River estuary, North Carolina, and maintained under constant conditions in the laboratory.

2. Swimming speed increases during rising tide with a maximum several hours after low tide, and decreases to a minimum about 2 hr after high tide. It is argued that this pattern is the basis of the endogenous tidal vertical migration, in which the larvae ascend on rising tides and descend on falling tides.

3. The tidal rhythm in phototaxis consists of an increase in negative responsiveness which is strongest around the middle of the rising tide, and a decline to a minimum during falling tides. Positive phototaxis shows the opposite pattern. There is no convincing evidence that phototaxis participates in vertical migration. It is hypothesized that the phototactic pattern represents a tidal rhythm in a shadow response used for predator avoidance.

LITERATURE CITED

Cronin, T. W., 1979. *Factors contributing to the retention of larvae of the crab* Rhithropanopeus harrisii, *in the Newport River estuary, North Carolina.* Ph.D. Thesis, Duke University. 206 pp.

Cronin, T. W., and R. B. Forward, Jr., 1979. Tidal vertical migrations: an endogenous rhythm in estuarine crab larvae. *Science,* **205**: 1020–1022.

Forward, R. B., Jr., 1974a. Phototaxis by the dinoflagellate *Gymnodinium splendens* Lebour. *J. Protozool.,* **21**: 312–315.

Forward, R. B., Jr., 1974b. Negative phototaxis in crustacean larvae: possible functional significance. *J. Exp. Mar. Biol. Ecol.,* **16**: 11–17.

Forward, R. B., Jr., 1976. A shadow response in a larval crustacean. *Biol. Bull.,* **151**: 126–140.

Forward, R. B., Jr., 1980. Phototaxis of a sand-beach amphipod: Physiology and tidal rhythms. *J. Comp. Physiol.,* **135**: 243–250.

Forward, R. B., Jr., and J. D. Costlow, Jr., 1974. The ontogeny of phototaxis by larvae of the crab *Rhithropanopeus harrisii*. *Mar. Biol.,* **26**: 27–33.

Forward, R. B., Jr., and T. W. Cronin, 1978. Crustacean larval phototaxis: Possible functional significance. Pp. 253–261 in O. S. McLusky and A. J. Berry, Eds., *Physiology and Behavior of Marine Organisms, 12th European Symposium on Marine Biology.* Pergamon Press, Oxford.

Fuller, W. A., 1976. *Introduction to Statistical Time Series.* John Wiley and Sons. New York. 470 pp.

Latz, M. I., and R. B. Forward, Jr., 1977. The effect of salinity upon phototaxis and geotaxis in a larval crustacean. *Biol. Bull.,* **153**: 163–179.

Palmer, J. D., 1973. Tidal rhythms: the clock of the rhythmic physiology of marine organisms. *Biol. Rev.,* **48**: 377–418.

Pritchard, D. W., 1952. Salinity distribution and circulation in the Chesapeake Bay estuarine system. *J. Mar. Res.,* **11**: 106–123.

Sandifer, P. A., 1973. Distribution and abundance of decapod crustacean larvae in the York River estuary and adjacent lower Chesapeake Bay, Viriginia. 1968–1969. *Chesapeake Sci.,* **14**: 235–257.

Sandifer, P. A., 1975. The role of pelagic larvae in recruitment to populations of adult decapod crustaceans in the York River estuary and adjacent lower Chesapeake Bay, Virginia. *Estuarine Coastal Mar. Sci.,* **3**: 269–279.